

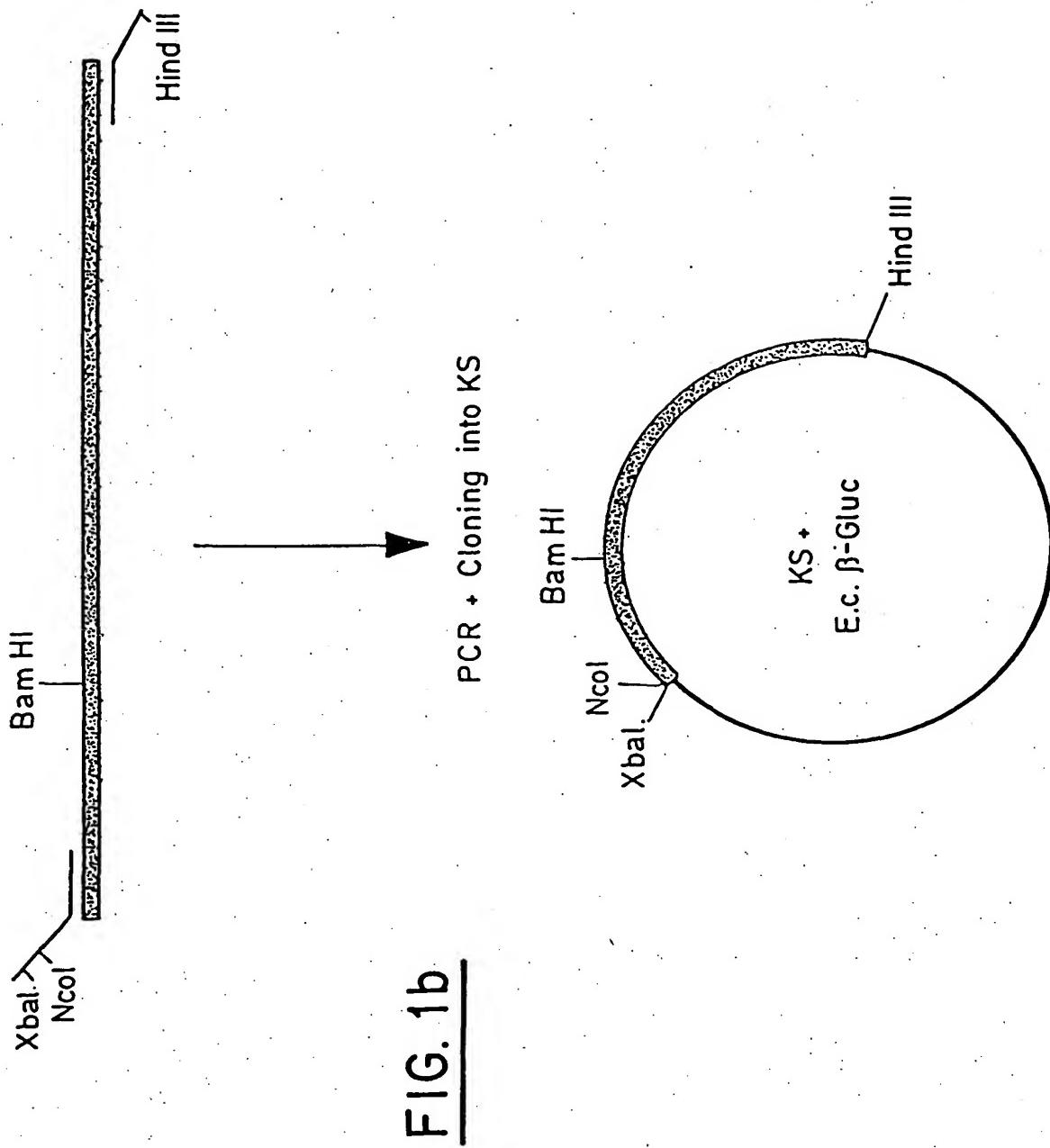
Martin OPPER et al.
"CYTOPLASMIC EXPRESSION OF ANTIBODIES,
ANTIBODY FRAGMENTS AND ANTIBODY
FRAGMENT FUSION MOLECULES IN E. COLI"
Attorney Docket No.: 38005-0183

FIG. 1a

E.c. β -Gluc for: 5' AAG CTT TCA TTG TT GCC TCC CTG CTG CGG 3'

E.c. β -Gluc back: 5' TCT AGA CCA TGG TAC GTC CTG TAC AAA CCC CA 3'

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FIG. 2a

Fab HC for: 5' GAA TIC CAT GGA ACC AGA ACC GAG CTC AAC TCT N2
link
CTT GTC CAC CTT GGT GTT 3'
C H 1

Fab HC back: 5' TCT AGA TAA CGA GGG CAA AAAAATG GAG GTC CAA CTG CAG N2b
SD V H
GAG AGC 3'

FIG. 2b

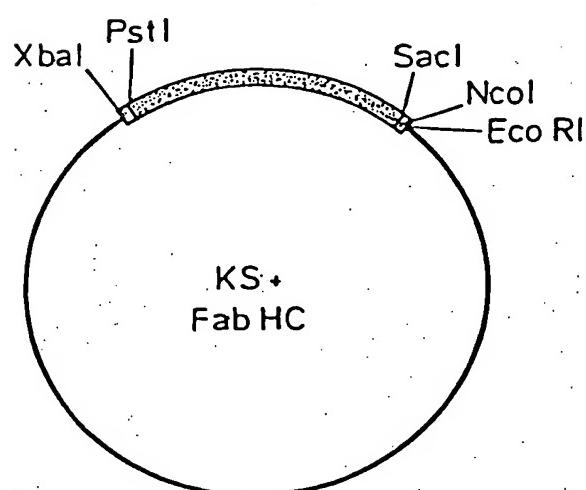
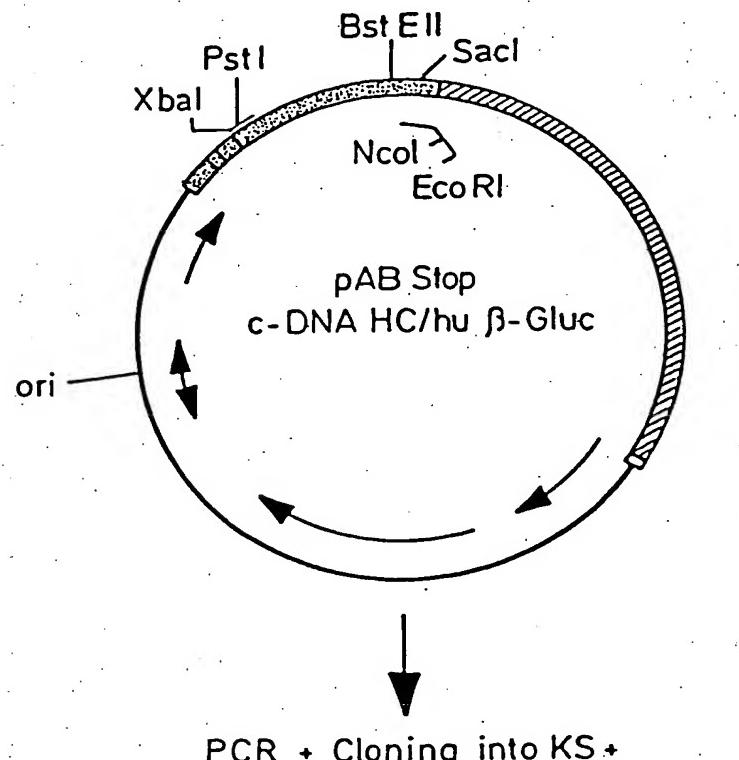
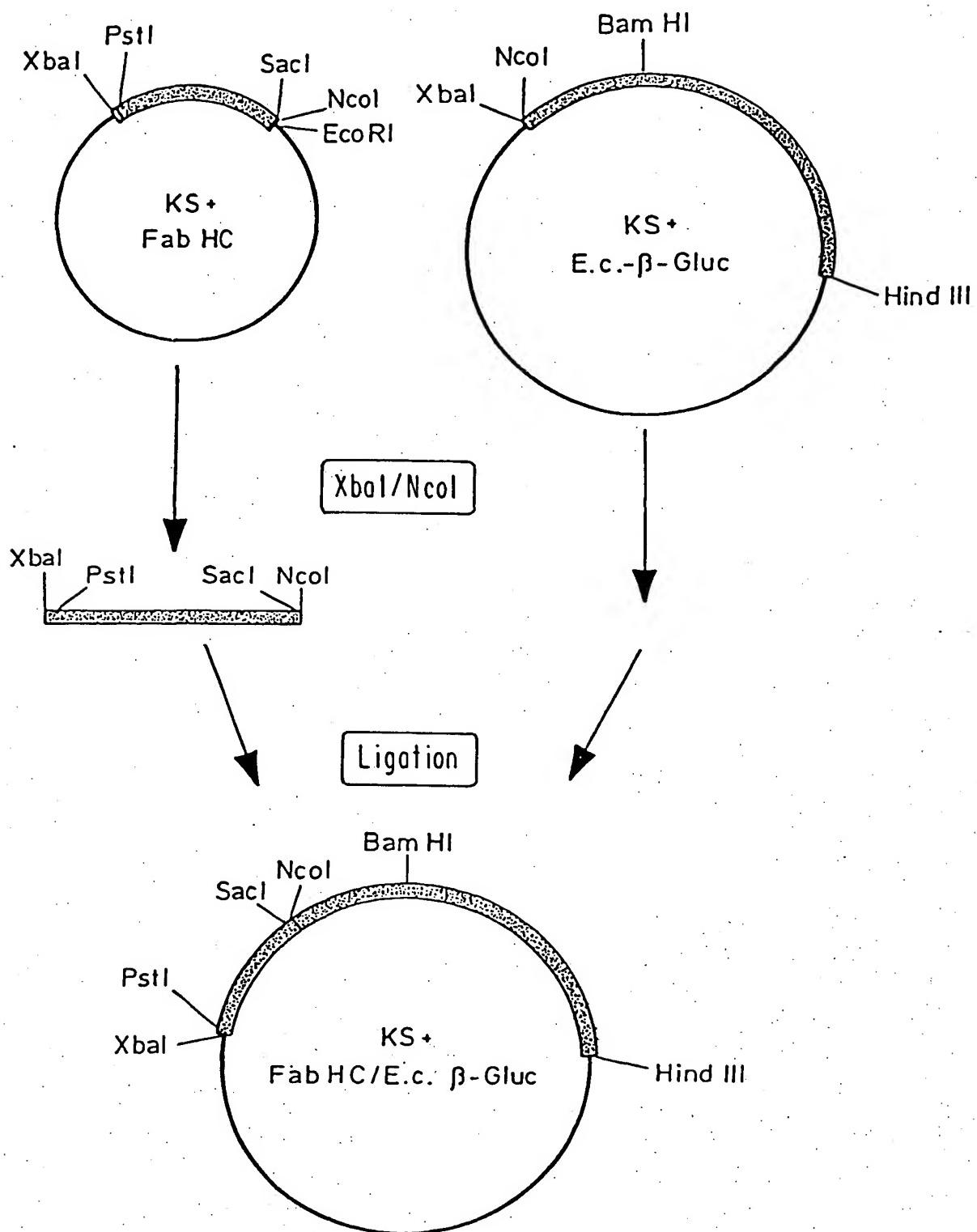


FIG. 3



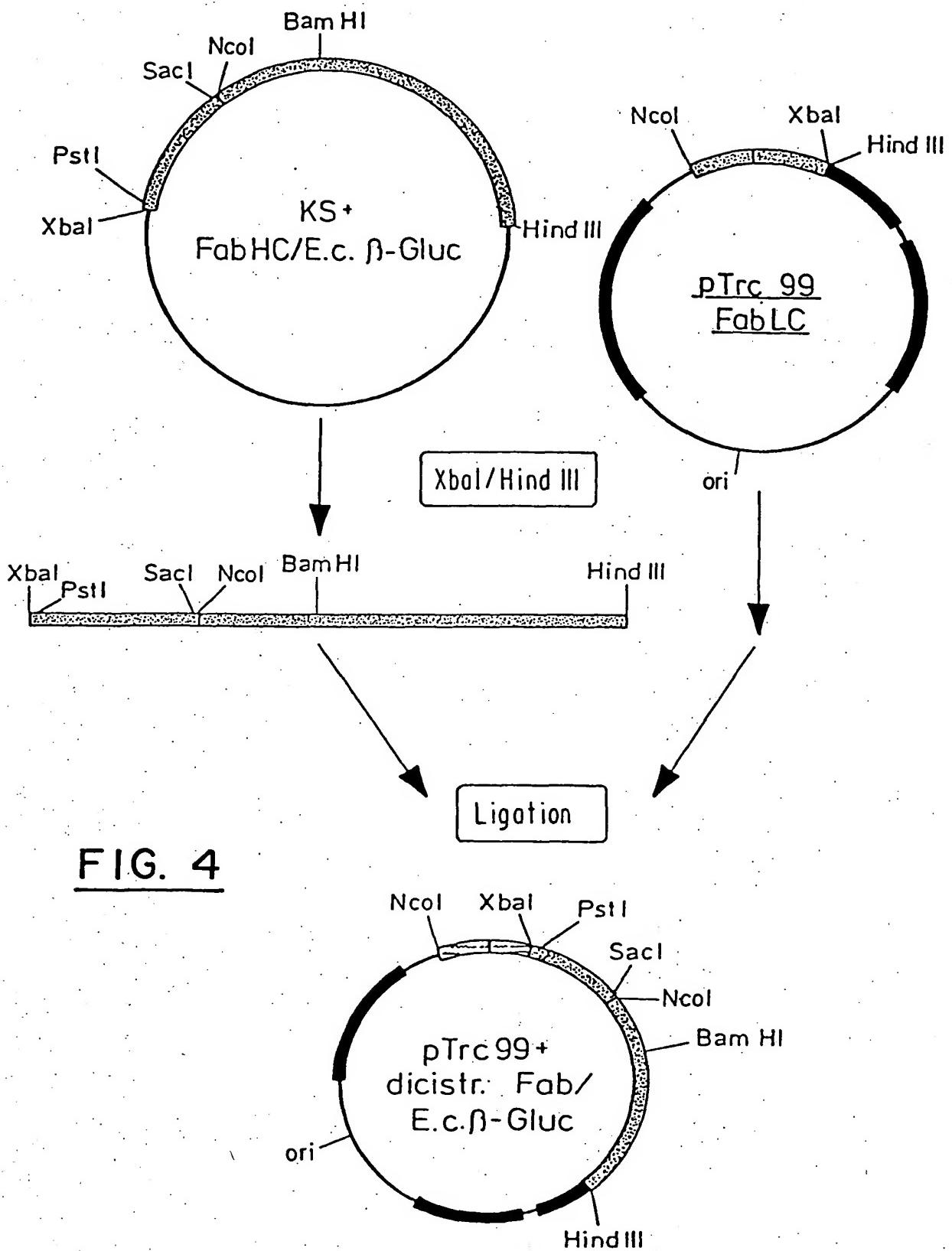


FIG. 4

431/26 hum dicistr. Fab / E.c.β - Glucuronidase in pTrc 99

FIG. 5a

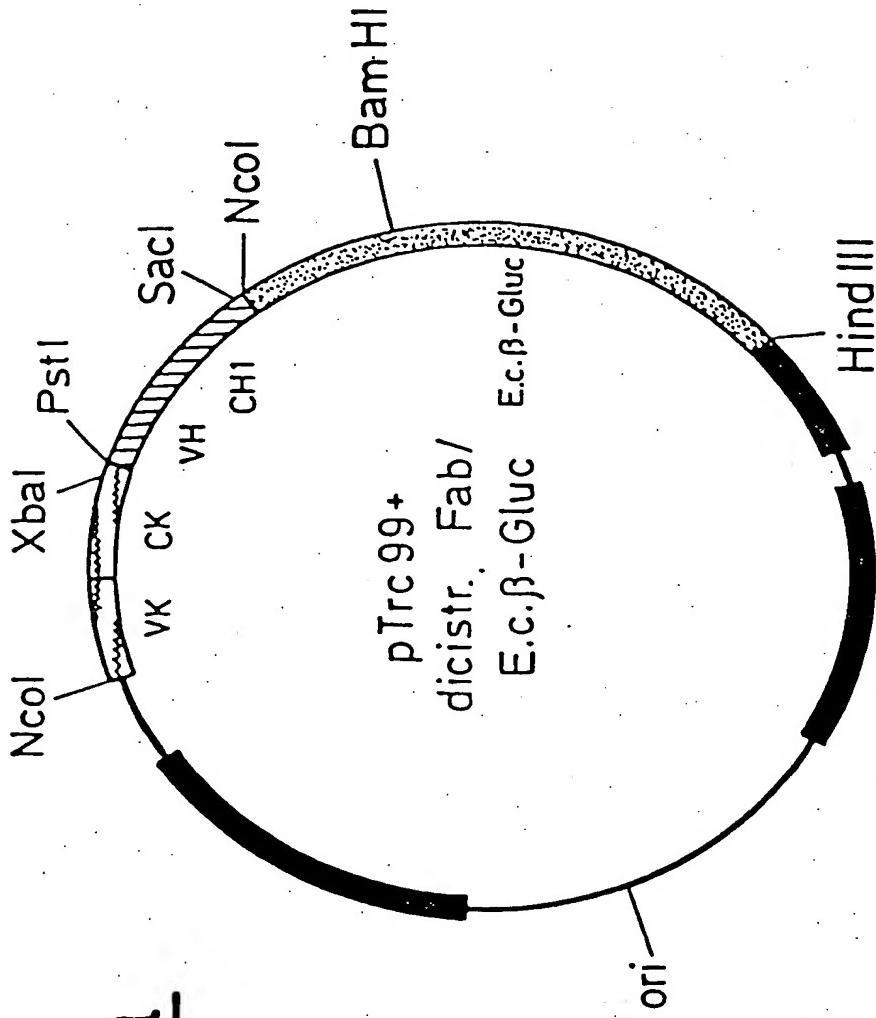


FIG. 5b

N_{co}l

1 CC ATG GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT GAC AGA
11^o Asp 11^o Glu Met Thr Glu Ser Pro Ser Ser Leu Ser Ala Ser Val Glu Asp Arg NS

60 CTG ACC ATC ACC TGT ACT ACC AGC TCG AGT GTA AGT TAC ATG CAC TGG TAC CAG CAG AAG
20^o Val Thr 11^o Thr Cys Ser Thr Ser Ser Val Ser Tyr Met His Trp Tyr Glu Glu Lys

120 CCA GGT CCA AAG CTG CTG ATC TAC AGC ACA TCC AAC CTG GCT TCT GGT GTG CCA VK
40^o Pro Glu Lys Ala Pro Lys Leu Leu 11^o Tyr Ser Thr Ser Asn Leu Ala Ser Glu Val Pro

180 AGC AGA TTC AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC CTC CAG
60^o Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr 11^o Ser Ser Leu Glu

240 CCA GAG GAC ATC GCC ACC TAC TAC TGC CAT CAG TGG AGT AGT TAT CCC ACC TTC GGC CAA
80^o Pro Glu Asp 11^o Ala Thr Tyr Tyr Cys His Glu Trp Ser Ser Tyr Pro Thr Phe Glu Glu

300 GGG ACC AAG GTG GAA ATC AAA CGT ACT GTC GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA
100^o Glu Thr Lys Val Glu 11^o Lys Arg Thr Val Ala Ala Pro Ser Val Phe 11^o Phe Pro Pro

FIG. 5c

360 TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG CAA AAC TAC TAT
120> Ser Asp Glu Gin Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr CK

420 CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG
140> Pro Arg Glu Ala Lys Val Glu Trp Lys Val Asp Asn Ala Leu Glu Ser Gly Asn Ser Glu

480 GAG AGT GTC ACA GAG CAG GAC ACC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTC ACG
160> Glu Ser Val Thr Glu Glu Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr XbaI

540 CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC
180> Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Glu Gly

600 CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG CGA GAG TGT TAG TCTAGATAACGAGGGCAA
200> Leu Ser Ser Pro Val Thr Lys Ser Ser Phe Asn Arg Gly Glu Cys ... PsII

664 AA ATG GAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CCT GTC AGA CCT AGC CAG ACC CTC
1> Met Glu Val Glu Leu Glu Ser Gly Pro Glu Val Arg Pro Ser Glu Thr Leu

FIG. 5d

723 ACC CTG ACC TGC ACC GTC TCT CCC TTC ACC ATC ACC AGT CCA TAT CGT TAC AGC TGG CAC TGG TCG GTC
20▶ Ser Leu Thr Cys Thr Val Ser Gly Phe Thr 116 Ser Ser Gly Tyr Ser Trip His Trip Val
VH

783 AGA CAG CCA CCT CGA CGA CGT CTT GAG TCG ATT CGA TAC ATA CAG TAC AGT GGT ATC ACT
40▶ Arg Glu Pro Pro Gly Arg Gly Leu Glu Trip 116 Gly Tyr 116 Glu Tyr Ser Gly 116 Thr
60▶ Asn Tyr Asn Pro Ser Leu Lys Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Glu
903 TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GGC GAC ACC GCG GTC TAT TAT TGT GCA AGA
80▶ Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Val Tyr Tyr Cys Ala Arg
963 GAA GAC TAT GAT TAC CAC TGG TAC TTC GAR GTC TGG GGT CAA CCC AGC CTC GTC ACA GTC
100▶ Glu Asp Tyr Asp Tyr His Trip Tyr Phe Asp Val Trip Gly Glu Val Val Thr Val Val
1023 ACA GTC TCC TCA CCT RCC ACC AAG GCG CCA TCG GTC CCC CTC GCG CCC TCC TCC ACC
120▶ Thr Val Ser Ser Ala Ser Thr Lys Glu Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1083 AGC ACC TCT GCG GCG ACA GCG CCC CTC GCG GTC CTC GAC TAC TCC CCC GAA CGG
140▶ Ser Thr Ser Gly Gly Thr Ala Ala Leu Glu Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro

CH1

FIG. 5e

1143 GTC ACG GTC TCG TCG AAC TCA GGC CCC CTG ACC AGC GGC GTG CAC ACC TTC CGG CCT GTC
160> Val Thr Val Ser Tri Asn Ser Tri Val His Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val

1203 CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTC GTC ACC GTC CCC TCC AGC ACC AGC TTC
180> Leu Glu Ser Ser Gly Leu Tyr Ser Val Ser His Lys Pro Ser Asn Thr Lys Val Asp Lys

1263 GGC ACC CAG ACC TAC ACC TCC AAC GTG ATC CAC NAG CCC AGC AAC ACC AGC GTG GAC AAG
200> Glu Thr Thr Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
link

Ncol
1323 AGA GTC GAG CTC CGT TCT GGT TCC ATG GTC CGT CCT GTC GAA ACC CCA ACC CCT
220> Arg Val Glu Leu Gly Ser Gly Ser Met Val Arg Pro Val Glu Thr Pro Thr Arg

1383 GAA ATC AAA AAA CTC GAC GGC CTG TGG GCA TTC AGT CTG GAT CCC GAA AAC TGT CGA ATT
240> Glu Ile Lys Lys Leu Asp Glu Leu Tri Ala Phe Ser Leu Asp Arg Glu Asn Cys Gly Ile

1443 GAT CAG CGT TGG TGG GAA AGC GGC TTA CAA GAA AGC CGG CGG AAC GTC TGG TAT CAG
260> Asp Glu Arg Tri Tri Glu Ser Ala Leu Glu Ser Arg Ala Ile Ala Val Pro Glu Ser

1503 TTT AAC GAT CAG TTC GCC GAT GCA GAT ATT CGT ATT TAT GCG GGC AAC GTC TGG TAT CAG
280> Phe Asn Asp Glu Phe Ala Asp Ile Arg Asn Tyr Ala Glu Asn Val Tri Tyr Glu E.C. β-Gluc

FIG. 5f

CGC GAA GTC TTT ATA CCG AAA CGT TCG GCA GCC CAG CGT ATC GTG CTG CGT Trp GAT GCG
300 Arg Glu Val Phe Ile Pro Lys Glu Trp Ala Gly Gln Arg Ile Val Leu Arg Phe Asp Ala

1623 GTC ACT CAT TAC GGC MAI GTG TGG AAT CAG GAA GTG ATG GAG CAT CAG GCC
320 Val Thr His Tyr Glu Lys Val Trp Val Asn Asn Glu Val Met Glu His Glu Gly Glu

1163 TAT ACG CCA TTT GAA CCC GAT GTC ACG CCG TAT GTT ATT GCC GGG AAA AGT GTA CGT ATC
 340 ▶ Tyr Thr Pro Phe Glu Ala Asp Val Thr Pro Tyr Val Ile Ala Glu Lys Ser Val Arg. Ile Thr
 11743 ACC GTG AAC AAC GTG CAG ACT ATC CCG CCG GGA ATG GTG ATT ACC
 360 ▶ Thr Val Cys Val Asn Asn Glu Leu Asn Trp Glu Thr Ile Pro Pro Gly Met Val Ile Thr

11803 GAC GAA AAC CCC AAG CAG TCT TAC TTC CAT AAT TTC AAC TAT GCC CGG ATC
380 Asp Glu Asn Glu Lys Lys Ser Tyr Phe His Asn Phe Asn Tyr Ala Gly Ile

1183 CAT CGC GTA ATG CTC TAC ACC AAC TCG GTC GAC GAT ATC ACC GTC GTC
4001 His Arg Ser Val Met Leu Tyr Thr Pro Asn Thr Tri P Val Asp Asp Ile Thr Val Val

11923 ACG CAT GTC CCG CAA GAC TGT AAC CAC CGC TCT GTT GAC TCC CAG GTC GTC CCC AAT GGT
420) Thr His Val Ala Glu Asp Cys Asn His Ala Ser Val Asp Thr Glu Val Ala Asn Glu

FIG. 5g

1983 GAT GTC ACC GTT GAA CTG CGT GAT GCG GAT CAA CAG GTC GTC GCA ACT GGA CAA GGC ACT
440> Asp Val Ser Val Glu Leu Arg Asp Ala Asp Glu Glu Val Val Ala Thr Glu Glu Glu Thr

2043 AGC GGG ACT TTG CAA GTG GTG ATT CCG CAC CTC TCG CAA CCG CGT GAA GGT TAT CTC TAT
460> Ser Glu Thr Leu Glu Val Val Asn Pro His Leu Trp Glu Pro Glu Glu Tyr Leu Tyr

2103 GAA CTG TCC GTC ACA GCC AAA AGC CAG ACA GAG TGT GAT ATC TAC CCG CTT CCC GTC GGC
480> Glu Leu Cys Val Thr Ala Lys Ser Glu Thr Glu Cys Asp Ile Tyr Pro Leu Arg Val Glu

2163 ATC CGG TCA GTG GCA GTG AAG CGC GAA CAG TTC CTG ATT AAC CAC AAA CCG TTC TAC TTT
500> Ile Arg Ser Val Ala Val Lys Glu Glu Glu Phe Leu Ile Asn His Lys Pro Phe Tyr Phe

2223 ACT GGC TTT CGT CGT CAT GAA GAT GCG GAC TTA CGT GGC AAA CGA TTC GAT AAC GTC GTC
520> Thr Glu Phe Glu Arg His Glu Asp Ala Asp Leu Arg Glu Lys Glu Phe Asp Asn Val Leu

2283 ATG GTG CAC GAC CAC GCA TTA ATG GAC TGG ATT GGG GCC AAC TCC TAC CGT ACC TCG CAT
540> Met Val His Asp His Ala Leu Met Asp Trp Ile Glu Ala Asn Ser Tyr Arg Thr Ser His

FIG. 5h

2343 TAC CCT TAC GCT GAA GAG ATG CTC GAC TCG GCA GAT GAA CAT GCC ATC GTG GTG ATT GAT
560> Tyr Pro Tyr Ala Glu Glu Met Leu Asp Trp Ala Asp Glu His Glu Ile Val Val Ile Asp

2463 GAA ACT GCT GCT GTC GGC GGC TTT AAC CTC TCT TTA GGC ATT GGT TTC GAA GCG GGC AAC AAG
580> Glu Thr Ala Val Glu Phe Asn Leu Ser Leu Glu Ile Glu Phe Glu Ala Glu Asn Lys
600>

2523 CCC ATT AAA GAG CTG ATA GCG CGT GAC AAA AAC CAC CCA AGC GTG GTG ATG TGG AGT ATT
620> Ala Ile Lys Glu Leu Ile Ala Arg Asp Lys Asn His Pro Ser Val Val Met Thr Ser Ile

2583 GCC AAC GAA CCC GAT ACC CGT CCG GAA TAT TTC GCG CCA CTC GCG GAA
640> Ala Asn Glu Pro Asp Thr Arg Pro Glu Ile Ala Arg Glu Tyr Phe Ala Pro Leu Ala Glu

2643 GCA ACG CGT AAA CTC GAC CCG ACG CGT CCG ACC TCC GTC AAT GTA ATG TTC TGC GAC
660> Ala Thr Arg Lys Leu Asp Pro Thr Arg Pro Ile Thr Cys Val Asn Val Met Phe Cys Asp

2703 CCT CAC ACC GAT ACC ATC AGC GAT CTC TTT GAT GTG CTG TGC AAC CGT TAT TAC CGA
680> Ala His Thr Asp Thr Ile Ser Asp Leu Phe Asp Val Leu Cys Leu Asn Arg Tyr Tyr Glu

2763 TCG TAT GTC CAA AGC GGC GAT TTG GAA ACG GCA GAG GTA CTG GAA AAA GAA CTT CTG
700> Trp Tyr Val Glu Ser Glu Thr Ala Glu Lys Val Leu Glu Lys Val Leu Glu Leu Leu

FIG. 5i

2823 TCC TCG CAG GAG AAA CTG CAT CAG CCG ATT ATC ATC ACC GAA TAC GCC GTG GAT ACG TTA
720> Ala Trp Glu Lys Leu His Glu Pro Ile Ile Thr Glu Tyr Glu Val Asp Thr Leu

2883 CCC CTC CAC TCA ATG TAC ACC GAC ATG TGG AGT GAA GAG TAT CAG TGT GCA TGG CTG
740> Ala Glu Leu His Ser Met Tyr Thr Asp Met Trp Ser Glu Glu Tyr Glu Cys Ala TriP Leu

2943 GAT ATG TAT CAC CGC GTC TTT GAT CGC GTC AGC GCC GTC GTC GGT GAA CAG GTA TGG AAT
760> Asp Met Tyr His Arg Val Phe Asp Arg Val Ser Ala Val Val Gly Glu Glu Val TriP Asn

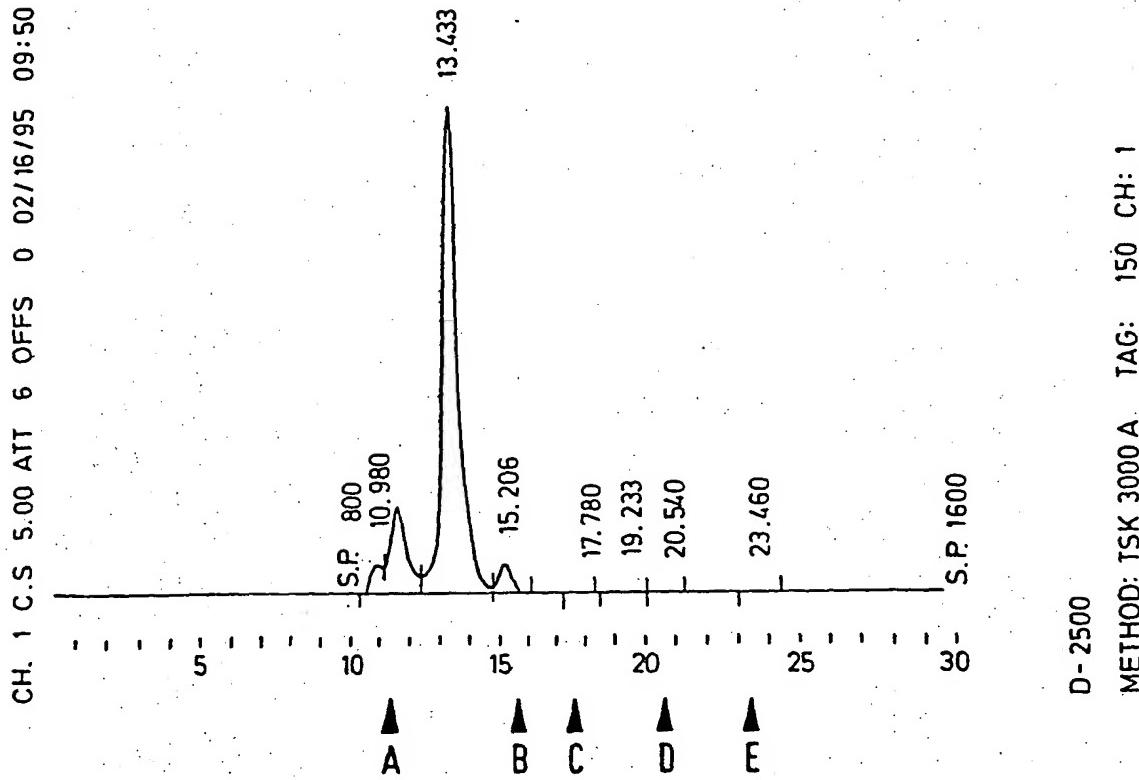
3003 TTC GCC GAT TTT GCG ACC TCG CAA CCC ATA TTT CGC CTT CGC CGT AAC AAG AAA GGG ATC
780> Phe Ala Asp Phe Ala Thr Ser Glu Ile Leu Arg Val Gly Glu Asn Lys Lys Glu Ile

3063 TTG ACT CGC GAC CCG AAA CCG AAG TCG GCG CCT TTT CTG CAA AAA CGC TGG ACT GGC
800> Phe Arg Asp Arg Lys Ser Ala Ala Phe Leu Glu Lys Arg TriP Thr Glu Lys Glu ...

HindIII
3123 ATG AAC TTC CGT GAA AAA CCG CAG GGA CGG AAA CAA TGA AGCTT
820> Met Asn Phe Glu Glu Lys Pro Glu Glu Lys Pro Glu Glu Lys Glu Lys Glu ...

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FIG. 6



TSK - 3000 Gel chromatography. Retention time of the fusion protein, 13.4 min.

- A: Thyroglobulin 670.000 D
- B: Gamma - Globulin 158.000 D
- C: Ovalbumin 44.000 D
- D: Myoglobin 17.000 D
- E: Vitamin B-12 1.350 D